ship in the department of pharmacology. The fellowship will aid in training pharmacologists and in the conduct of research, both fundamental and applied.

THE Moore School of Electrical Engineering of the University of Pennsylvania will be enlarged at a cost of nearly $125,000. A contract awarded by the university calls for construction of a third floor which will add 13,000 square feet of laboratory space. The additional laboratory area will be adapted for research in various phases of electronics, such as television and industrial control, and will be used also for the investigation of problems related to electrical machinery.

SPECIAL ARTICLES

SUSCEPTIBILITY OF THE YOUNG WHITE MOUSE (MUS MUSCULUS) TO EXPERIMENTAL LEPTOSPIROSIS

IN 1941 Larson reported[1] that white mice (*Mus musculus*) about 3 weeks of age were uniformly susceptible to experimental infection with *Leptospira icterohemorrhagiae* by practically all routes of infection. Inoculation was followed by the development of icteric and hemorrhagic lesions typical of this infection and by death within two weeks. Subsequently, the same worker perfected a diagnostic protection test[2] for leptospirosis based on the extreme sensitivity of young white mice to infection. Inasmuch as the guinea pig, the diagnostic animal now used, is less susceptible and more expensive than mice, the advantages of the new test are obvious. It was therefore considered worthwhile, in the course of other studies on experimental leptospirosis, to determine whether our strain of white mouse is susceptible to this infection.

For this purpose three cultures of *Leptospira icterohemorrhagiae* on Verwoort-Schuffner medium, strains labeled 626, 1653 and WRZ, were obtained from Dr. Larson, at the National Institute of Health, Bethesda, Maryland. Each of these strains had been passed by Larson through many generations of white mice and had killed practically 100 per cent. of injected animals. The mice used in our studies were a strain of *Mus musculus* which had been cage-inbred for many years in the Department of Bacteriology at the University of Minnesota. Unfortunately, no records are available revealing the origin of this strain.

Immediately upon receipt, each strain of spirochete was inoculated intraperitoneally in 0.5 ml amounts into 2 young white mice about 3 weeks old. One milliliter of each culture was subcultured on several tubes of Schuffner medium. As soon as subcultures revealed a heavy growth of organisms upon dark-field examination, they were used as inoculum in experiments. In order to test the virulence of the cultures, animals known to be susceptible to leptospirosis were inoculated. One milliliter was injected intraperitoneally into 2 deer mice and 2 young guinea pigs, and 0.5 ml by the same route into a young Syrian hamster.

One milliliter doses of culture were injected intraperitoneally and subcutaneously into young white mice between 2 and 3 weeks old, and 0.03 ml doses were injected intracranially. These inocula and routes of infection were also used in mice that had been inoculated intraperitoneally with 0.25 ml of a mixture of equal parts of benzol and olive oil 36 hours earlier. A third group of young mice was injected by the same routes and with the same volumes of leptospirae suspended in equal volumes of mucin prepared by Siler's method.[3] Another group of young mice were inoculated intraperitoneally with 0.5 ml of spirochetal culture 24 hours after establishment of a sterile inflammatory focus in the brain of each animal by intracranial inoculation of 0.03 ml of 5.5 per cent. starch solution in nutrient broth. Unless otherwise indicated, 3 mice were inoculated with each dose and by each route.

Daily cultures were made from the heart's blood of all animals for 3 weeks following inoculation. The mice were observed carefully daily for signs of generalized jaundice or hemorrhage and hyperemia of the bulbar conjunctivae. One, two and three weeks after inoculation, one animal from each group of 3 was killed by asphyxiation with gas. Subcultures, inoculations and tissue sections were made from the kidneys, livers and lungs of all animals, and also from the brains of animals inoculated intracranially or intraperitoneally following intracranial administration of starch solution. The sections were stained with hematoxylin and eosin.

All the inoculated guinea pigs and deer mice and the hamster developed typical signs of leptospirosis and succumbed within 10 days after injection. On the other hand, none of the injected white mice exhibited signs of frank leptospirosis and, in fact, none appeared sick. However, most of the blood cultures made from infected white mice were positive for about a week after subcutaneous and intraperitoneal inoculation, but were negative following intracranial injection. Organisms were isolated by animal inoculation

[1] C. L. Larson, *Pub. Health Rep.*, 56: 1546, 1941.
[2] *Ibid.*, 56: 1593, 1941.
[3] J. F. Siler, "Immunization to Typhoid Fever." Baltimore: Johns Hopkins Press, 1941.

from the blood, kidneys and liver of many mice during the first week, but they were isolated only from the kidneys of some mice during the second and third weeks after injection. Mild congestion of the liver and occasional hemorrhages in the kidneys and lungs were observed in sections of mouse tissues. The brains were normal except for a mild, mononuclear meningitis in a few instances. Multiple petechial hemorrhages in the lungs were the most common lesions observed. In the few cases in which sera taken from the mice after injection were pooled and tested, the several lots agglutinated live and killed leptospirae in titers ranging from 1:10 a week after inoculation to 1:30,000 3 weeks after injection.

After unsuccessful attempts to produce frank leptospirosis in young white mice by the methods described, it was decided to use younger mice and larger doses of organisms in the hope of overwhelming an immunologically-immature host with great numbers of organisms. Accordingly, 5 mice several days old were inoculated intraperitoneally with 1.5 ml of a heavy suspension of strain 1,653. These mice were observed and examined exactly as were the more mature animals. The results of these studies were similar to the results of the studies on older individuals. None of the immature mice succumbed to the infection, although all of them eventually became carriers of the organism, harboring them in their kidneys. Examination of tissue sections disclosed that lesions the same as those seen in the older mice occurred more frequently in the younger individuals. Agglutination tests performed on 2 sera collected 3 weeks after injection yielded titers of 1:300 and 1:100 against the spirochete.

DISCUSSION

In the present study, in which the same strains of spirochete used by Larson[1,2] were employed, our strain of young white mouse (*Mus musculus*) did not develop fatal leptospirosis. Although the animals that did become infected manifested some of the early pathological changes characteristic of leptospirosis and harbored the organisms in their kidneys, they did not succumb to the infection. Several methods used by other investigators either to enhance the virulence of the infective agent (suspension in mucin[4]), or to interfere with the defensive reactions of the host to infection (inoculations of benzol[5] and starch[6]) have failed to influence the course of experimental infection of the young white mouse with leptospirae. Inoculation of overwhelming doses of organism into immunologically-immature individuals was also without effect. Packchanian[7] has already shown that there are differences of varying degree in the susceptibility of different species of mice and rats to leptospirosis. A short while after these experiments were completed a paper by Das Gupta[8] appeared. This investigator did not use special methods to facilitate infection. He was unsuccessful in infecting a number of 3-week-old white mice (*Mus musculus*) with *Leptospira icterohemorrhagiae*. Control guinea pigs injected with the same cultures succumbed to typical and fatal infections.

The results of this study, combined with those of Larson on the susceptibility of his strain of *Mus musculus* to fatal infection, and the negative results of Das Gupta suggest that in addition to differences between species there are differences within species as to resistance to leptospirosis. A genetic difference between strains within a species has already been observed in mice with respect to resistance to mouse typhoid[9] and mammary carcinoma.[10] The mechanism or basis of the genetic differences in resistance to leptospirosis has not been fully elucidated. However, other studies by one of us[11] suggest that the humoral rather than the cellular defensive reactions of the host are the important factor involved. It is possible that subsequent studies may show a significantly greater rate of production of lytic antibodies in resistant strains of white mice than is found in more susceptible strains.

SUMMARY

The failure of even special methods to induce infection indicates that not all strains of young white laboratory mice (*Mus musculus*) are uniformly susceptible to fatal infection with *Leptospira icterohemorrhagiae*. Some strains of this species develop early pathological changes characteristic of the infection and harbor the organism in their kidneys, but do not succumb. Therefore not all strains of white mice are suitable for use in the diagnostic mouse protection test for leptospirosis described by Larson.[2]

ABRAM B. STAVITSKY
ROBERT G. GREEN
DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY,
MEDICAL SCHOOL,
UNIVERSITY OF MINNESOTA

[4] W. J. Nungester, L. F. Jourdonais and A. A. Wolf, *Jour. Inf. Dis.*, 59: 11, 1936.
[5] A. R. Rich and C. M. McKee, *Johns Hopkins Hosp. Bull.*, 54: 277, 1934.
[6] W. L. Sawyer and W. Lloyd, *Jour. Exp. Med.*, 54: 533, 1931.
[7] A. Packchanian, *Pub. Health Rep.*, 55: 1389, 1940.
[8] B. M. Das Gupta, *Ind. Med. Gaz.*, 77: 284, 1942.
[9] J. W. Gowen and M. L. Calhoun, *Jour. Inf. Dis.*, 73: 40, 1943.
[10] J. J. Bittner, *Cancer Research*, 1: 793, 1941.
[11] A. B. Stavitsky, *Jour. Exp. Med.*, manuscript in progress.